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THE EFFECT OF GALACTOSE FEEDING ON THE TOPOLOGICAL DISTRIBUTION OF PROTEIN AND LIPID IN RAT LENSES

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Lu-Ku Li, Raúl Chiesa, Dong-Sheng Dong and S.-C. Joseph Fu (1991) The effect of galactose feeding on the topological distribution of protein and lipid in rat lenses. *Bull. Inst. Zool., Academia Sinica* **30**(3): 237-246. Individual lenses from rats fed with chow alone (control), chow containing 50% glucose or chow containing 50% galactose were mechanically separated into an outer cortex (OCX), an inner cortex (ICX) and a nucleus (NU) fraction. Compared to the control, the glucose diet resulted in a smaller increase in lens dry weight (mostly protein) while the galactose diet resulted in no significant increase in lens dry mass during the 14-day experimental period. A significant difference in lens dry weight of the control and the galactose-fed animals was detected on the 4th day of the experiment. As early as one day on the galactose diet, an increased amount of lens dry weight was recovered in the outermost OCX fraction.

Both the cholesterol and phospholipid concentration in the control lenses showed a cortex to nucleus decrease from 5.5 to 4 and 15 to 5 μg/mg dry weight, respectively. The extraction of increasing amount of lens fibers in the OCX fractions of animals on the galactose diet resulted in lower than normal lipid levels observed in these fractions. The lens phospholipid composition of animals on the different diets was similar. Phosphatidylethanolamine, phosphatidylserine, phosphatidylcholine and sphingomyelin were the major components. The serum cholesterol level of the galactose-fed rats, 72 mg/dl, was higher than that of the control, 61 mg/dl. Since a high cholesterol level is known to inhibit cell growth and differentiation, the observed difference in lens weight of the galactose-fed animals may reflect a disruption in normal fiber cell development.

Key words: Rat lens, Galactose diet, Protein distribution, Cholesterol, Phospholipid, Lens growth, Serum chemistry.

Profound changes in lens biochemistry and morphology have been reported to occur during the early stages of cataractogenesis in galactose-fed rats (Kinoshita *et al.*, 1962; Kuwabara *et al.*, 1969). The increased water content of the lens due to the accumulation of polyol in the cells leads to alterations in the plasma membrane function and a consequent disturbance of the distribution of ions. At a very early stage, within 24 h of galactose

feeding, a large decrease in such metabolic mediators as glutathione (Reddy *et al.*, 1976) and prostaglandins (Keeting *et al.*, 1987) has been demonstrated. In addition, disorganization of the germinative epithelium has been observed (Cotlier, 1962) which suggests an interruption in the normal development of the lens fibers. If the condition persists, irreversible alteration of the plasma membrane occurs causing disruption of the fiber cells and lens opacification.

Since the comparative analysis of tissues from concentric layers of individual lenses has been successfully applied in this laboratory to characterize aging and developmental changes in lens fiber cells (Li *et al.*, 1987), the same approach was undertaken to study changes occurring in the lenses of rats on a 50% galactose diet. Successive layers of lens fibers obtained by vortexing individual lenses in water were examined. An increase in the amount of lens tissue recovered in the outermost fraction, OCX, indicative of a loss of lens mechanical integrity, was detected in rats as early as 24 h on the galactose diet. Concomitant changes in lens lipid distribution and in serum cholesterol and glucose levels were also observed in the animals. All these alterations appear to be the result of a systemic metabolic disturbance produced in the rat by the high galactose diet.

MATERIALS AND METHODS

Animals

Young, male, Sprague-Dawley rats (approximately 22 days of age) were purchased from Taconic Farms Inc., Germantown, New York. After a 3-day acclimation period, the animals were fed one of the following diets: Purina Rodent Laboratory Chow (Purina Ralston, St. Louis, MO., USA) alone as controls, chow containing 50% glucose or chow containing 50% galactose.

Fractionation of individual lenses

At the predetermined intervals, the animals were sacrificed, blood samples taken and the lenses removed. Individual lenses were decapsulated and each placed in a 95×16 mm polypropylene centrifuge tube with 0.5 ml water and stirred in a Geneie Mixer (Fisher Scientific, Springfield, NJ) for 20 sec at the maximal setting. The suspension containing the outer cortical fibers (OCX) was collected. Another 0.5 ml of water was added to the tube containing the remainder of the lens and stirred for 2 min at the same setting to isolate the inner cortical fibers (ICX). The remaining tissue in 0.5 ml water was taken as the nuclear fraction (NU). Each fraction was homogenized with a high speed tissue homogenizer (Ross Emulsifier, Ross and Son Co., Copaigue, NY) at 40% power for 20 sec under argon. Portions of the homogenate were removed for dry weight determination and the remainder was extracted for lipid.

Lipid extraction and analysis

Extraction of lipid was carried out with chloroform:methanol (2:1). Cholesterol was determined by the cholesterol oxidase assay (Boenringer, Indianapolis, IN). Phospholipid was estimated from the inorganic phosphate content of the lipid extracts. Individual phospholipids were identified by thin layer chromatography in one dimension using a two solvent system. The details of these procedures have been described previously (Li *et al.*, 1987; Li and So, 1987).

Dry weight determination

The dry weight of the lens was determined by heating 20 μl samples of

homogenates in microbalance aluminum pans (Cahn Instruments, Cerritos, CA) for 4-6 h at 110°C until a constant weight was reached (Li and So, 1987).

Serum glucose and cholesterol

Serum glucose was determined by an automated enzymatic procedure (Lloyd *et al.*, 1978). Total serum cholesterol levels were measured by an automated method of Zlatkis *et al.* (1953).

Statistical analysis

Statistical analyses were performed as prescribed in Sokal and Rohlf (1981). A computer program, Statgraphics (Statistical Graphics Corp., USA), was used with an IBM PC to facilitate the calculations.

RESULTS

Body weight and lens dry weight

At the beginning of the experiment, the average body weight of the rats was 65±12 g. By the end of the experiment (day 14), the control animals weighed 163±10 g. Significantly lower values were observed in rats on the glucose or galactose diets, 118±5 and 115±12 g (n=4, p=0.001), respectively.

The total lens dry weight of the control animals increased from 7.5±0.4 to 8.9±0.6 mg (n=4, p=0.007) during the 14-day experimental period (Fig. 1). During the same period, rats fed with the glucose diet showed a somewhat smaller increase in the total lens dry weight, from 6.8±0.9 to 8.2±0.6 mg (n=3, p=0.052). In contrast, in the galactose-fed animals, no significant difference in the total lens dry weight between day 1 and day 14 was found (7.3±0.3 and 7.0±0.5 mg, n=4). Moreover, when data from 3 of the 14-day galactose-fed rats having a dense nuclear opacity and large OCX (6.7±0.4 mg) were compared with those of day 1, a net decrease in lens dry weight was obtained (p=0.054). This decrease in the lens dry weight was more apparent in rats after 8 days of galactose feeding (5.6±0.8, n=4, p=0.006). Furthermore, when compared with control animals of the same age, the lens dry weight of the galactose-fed animals showed a statistically significant difference (p=0.018) as early as the 4th day of galactose feeding. The data suggest that, in addition to lens growth, other process(es) were

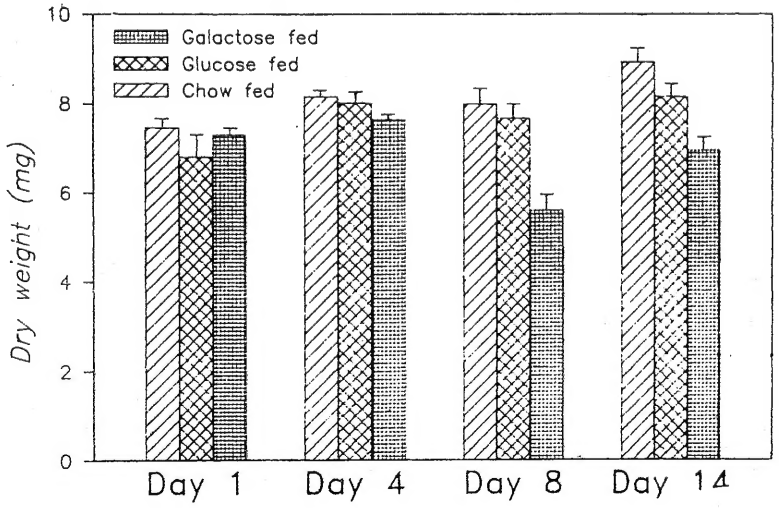

Fig. 1. The total lens dry weight was estimated from the sum of the dry weights of the OCX, ICX and NU fractions. Values are the average of 4 determinations each from one lens of one animal; bars represent the SD.

affected by the galactose diet (see Discussion Section).

Distribution of lens dry mass in individual lenses

During the 14-day experimental period, the relative amount of lens dry weight recovered in the OCX and NU fractions of the control lenses increased slightly while that of the ICX fraction decreased (Fig. 2; upper row). The distribution of fiber dry mass in individual lenses of rats on the glucose diet was similar to that of the controls (data not shown). After one day on the galactose diet, while the total lens dry weight remained the same as the controls, the relative size of the OCX fraction in 2 of the 4 rats accounted for 21% of the lens dry mass (Fig. 2; middle row). In the other 2 animals, the size of the OCX was 7% of the total, approximately the same as that of the control. The proportion of the OCX fraction increased further as the galactose feeding was continued and by the 14th day of the experiment, this fraction contained more than 70% of the lens dry mass in 3 of the 4 rats. These lenses had opaque nucleus and collapsed upon the removal of the capsule epithelium during the fractionation procedure. No lens material was recovered in the ICX fraction and the proportion of the NU fraction was greatly reduced from that found in the control lenses.

Distribution of cholesterol in individual lenses

The cholesterol concentration in the major lens fractions varied in the range of 4 to 6 μg/mg dry weight in both the control and the galactose fed animals (Fig. 3). Lenses from the 2 animals having a different lens weight distribution as

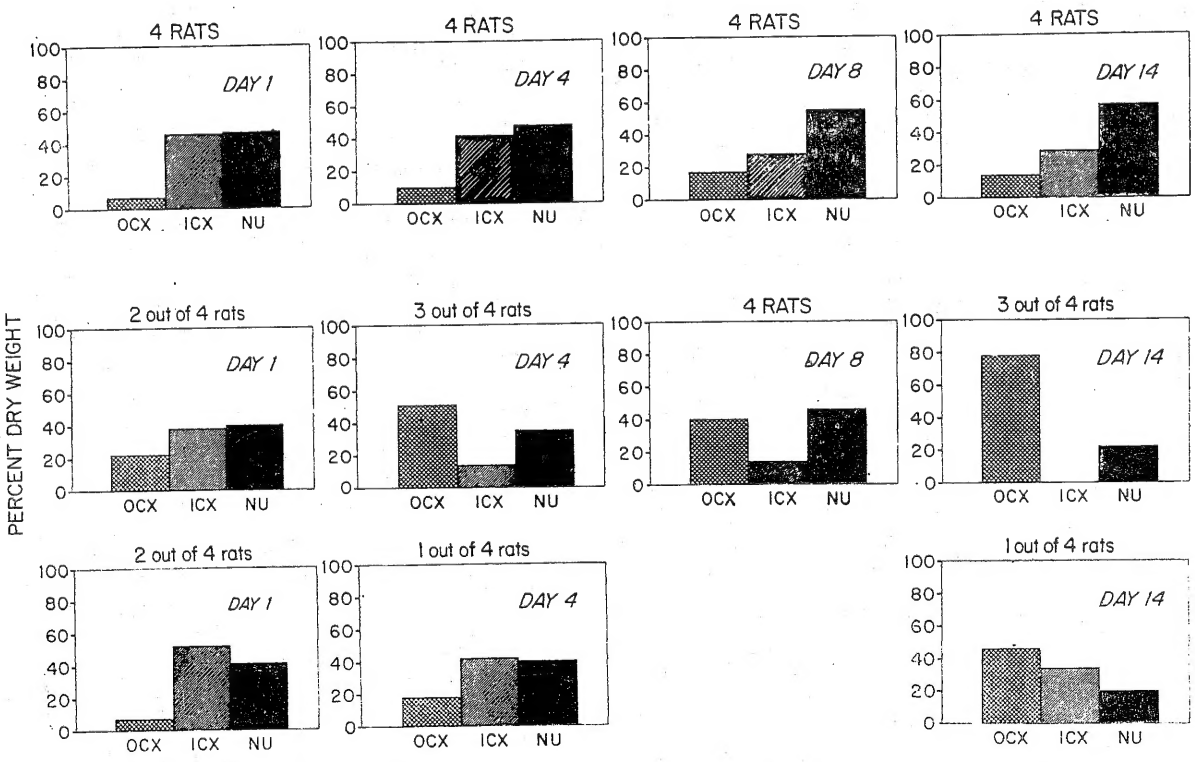

Fig. 2. The distribution of the lens dry weight in OCX, ICX and NU fractions of controls (upper row) and galactose-fed (middle and lower rows) rats. One lens from each animal was used, the data represent average values. The SD is less than 18% of the mean value.

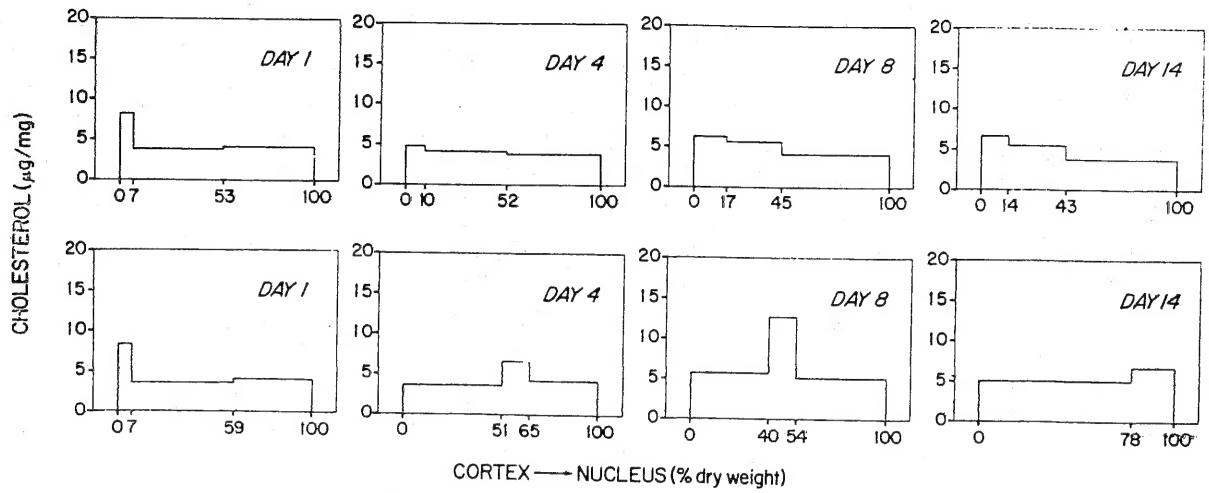

Fig. 3. Cholesterol concentration in the lens OCX, ICX and NU fractions of controls (upper row) and galactose-fed (lower row) rats, on the indicated day of the experiment. The location of each fraction is indicated by a horizontal line on a cortex to nucleus axis representing 0 to 100% of the total lens dry weight, where 0% is the surface and 100% the center of the lens. Abscissa values indicate the accumulative % of the lens dry weight. Ordinate values are the averages from single lenses from two or more animals. Lenses from the galactose-fed rats shown in Fig. 2, middle row were analyzed. The SD is less than 10% of the mean except for fractions smaller than 15% of the total lens weight for which the SD reaches 22%.

compared to the controls (day 1) or the majority of the animals (day 4, day 8 and day 14) on the galactose diet were analyzed. (Values from fractions less than 15% of the lens dry weight showed large experimental variations and may not be representative.) In the lenses of the control rats, except perhaps those

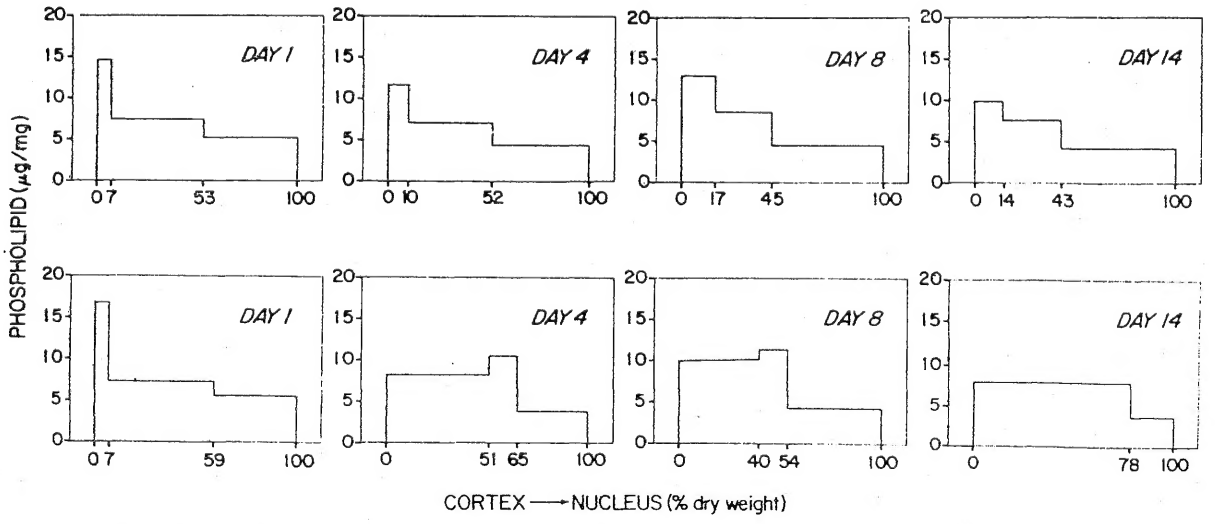

Fig. 4. Phospholipid concentration in the lens OCX, ICX and NU fractions of controls (upper row) and galactose-fed (lower row) rats. The same fractions in Fig. 3 were analyzed. See legend to Fig. 3. The SD is less than 10% of the mean.

from day 1 of the experiment, a cortex to nucleus decrease in cholesterol content was found, from approximately 5.5 to 4 μg/mg. In lenses from the galactose-fed animals, such a cortex to nucleus decrease in cholesterol content was not apparent. The OCX fractions containing a large proportion of the total lens showed cholesterol levels similar to those found in the NU fractions.

Distribution of phospholipid in individual rat lenses

The average phospholipid concentration in individual control lenses decreased from the cortex to the nucleus from approximately 15 to 5 μg/mg dry weight (Fig. 4). The OCX fractions containing the newly formed lens fibers always had the highest phospholipid level. After the first day, the phospholipid concentration of the large OCX fraction of lenses from the majority of the galactose-fed animals was consistently lower than that observed in the corresponding control fractions. This decrease in the cortical phospholipid concentration reflects the presence of deeper fiber cells of low phospholipid content recovered as the OCX fraction. The nuclear levels found in lenses of both control and experimental rats were very similar.

Phospholipid composition of the rat lens fiber

The phospholipid compositions of the

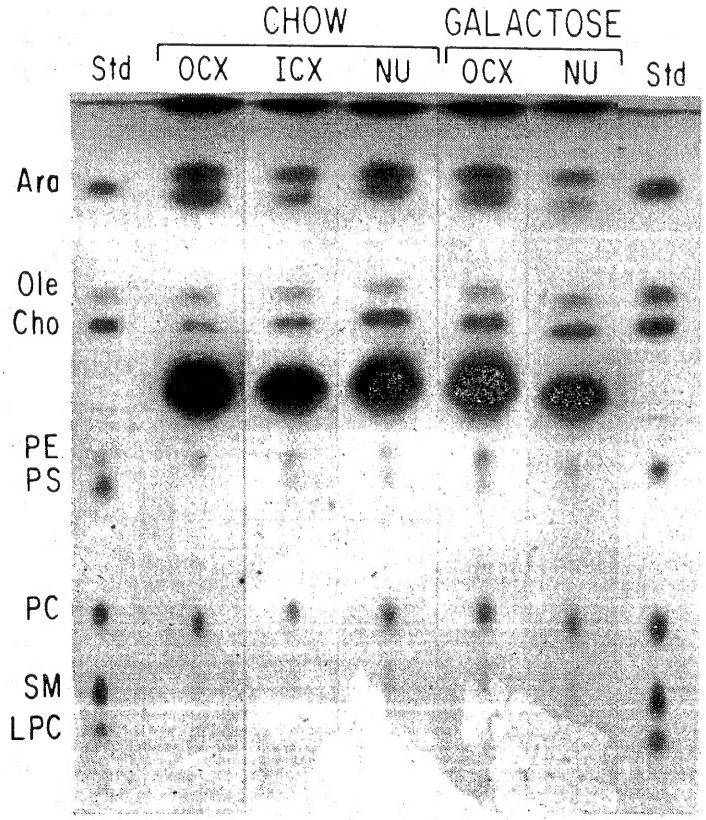

Fig. 5. Thin layer chromatography of lipid extracts from lens fractions of rats after 14-days on the control and galactose containing diets. Approximately 5 μg phospholipid was applied to each lane with 0.5 μg oleyl alcohol (Ole) as the internal standard. Standards used are: Ara, arachidic acid; Cho, cholesterol; PE, phosphatidylethanolamine; PS, phosphatidlylserine; PC, phosphatidylcholine; SM, sphingomyelins; LPC, lyso-PC.

Table I
Average cholesterol and glucose levels (mg/dl, N=4) of rats on the chow (CONTR), glucose (GLU) and galactose (GAL) diets. Values of Day 1-14 are averages of all data points,, N=16

	CHOLESTEROL				GLUCOSE		
	CONTR	GLU	GAL		CONTR	GLU	GAL
Day 1	52±3	45±11	67±8	Day 1	199±60	144±10	123±8
Day 4	64±7	58±11	58±11	Day 4	174±38	215±26	146±24
Day 8	63±21	78±5	75±17	Day 8	174±12	164±33	134±36
Day 14	65±12	65±8	88±8	Day 14	137±12	180±43	199±74
Day 1-14	(61±13)*	66±16	(72±15)*	Day 1-14	171±40	173±38	147±45

* p=0.037, N=16, Significance

rat lens lipid extracts analyzed by thin layer chromatography in one dimension developed with the two solvent system are shown in Fig. 5. The major phospholipids revealed are phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylcholine (PC) and sphingomyelin (SM). PC and PE are the most abundant phospholipids in these young rats. As exemplified by the 14-day data, the phospholipid compositions of the cortical and nuclear fibers in the control as well as in the experimental lenses are similar. The material migrating with the first solvent front represents isolation artifacts probably of protein and lipid complexes. The material migrating with the second solvent front was not identified.

Changes in serum cholesterol and glucose levels in galactose-fed rats

The serum cholesterol level in the control rats varies between 52 to 65 mg/dl during the experimental period of 14 days (Table 1). The average level over the entire experimental period was 61±13 mg/dl, which is significantly lower than that of the galactose-fed rat, 72±15 mg/dl (p=0.037). An intermediate cholesterol level of 66±16 mg/dl was found in rats on the 50% glucose diet. These results suggest that both glucose and galactose influence the lipid metabolism of the rat, yet they differ in their effectiveness and possibly the mechanism(s) involved.

The serum glucose level in the control animals showed a large variation, from 199 to 137 mg/dl during the experimental period. The average level over the entire experimental period was 171±40 mg/dl, which appeared higher than that of the galactose-fed animals, 147±45 mg/dl. However, the difference is not statistically significant. Animals on the glucose diet showed a serum glucose level comparable to that of the controls, 173±38 mg/dl. Similar results on serum cholesterol and glucose levels were obtained with a second experimental group of rats fed for 15 days.

DISCUSSION

Loss of lens dry weight and changes in its distribution in individual lenses

The significantly smaller body weights observed in both glucose- and galactose-fed rats as compared to that of the controls indicates a systemic alteration in the growth and development of these animals. Such an alteration is probably the consequence of a metabolic adaptation to the high sugar diets. Increased dietary glucose and fructose have been shown to cause basic changes in liver metabolism

in the rat (Bar-on and Stein, 1968). However, the "aged" appearance of the galactose-fed animals, the loss of body hair, the opacification of the lens, etc. suggest the involvement of additional factor(s) due to the galactose diet. The loss of lens mass during galactose feeding is generally understood on the basis of an accumulation of sugar alcohol, the osmotic disruption of lens cells and the loss of lens content (Kinoshita *et al.*, 1962).

Analyses of the distribution of tissue mass in individual lenses indicate an early loss of lens mechanical integrity during galactose feeding. This is consistent with the reported appearance of large extracellular cysts (Kuwabara *et al.*, 1969) and disorganization of the germinative zone in lens epithelium (Cotlier, 1962) during the early stages of galactose feeding. The "liquefaction" of the lens results in an increase in the amount of lens fiber material recovered in the OCX fraction. The increase can be detected as early as one day on the galactose diet. On the 4th day and thereafter, 40% or more of the total lens dry mass was found in this fraction. The inclusion of deeper fibers having a low lipid content could account for the lower than normal cholesterol and phospholipid contents found in the OCX fraction (Figs. 3 and 4).

Fiber cell membrane rigidity

The molar cholesterol/phospholipid ratio is a measure of the physical characteristics of the lens fiber membrane. From data in Figs. 3 and 4, the ratio of control rats was estimated to be approximately 0.8–1.0 in the outer cortex and 1.6–1.8 in the nucleus. This cortex to nucleus increase was mainly due to a corresponding decrease in phospholipid. In the galactose-fed rat, a somewhat higher ratio of 0.9–1.3 was observed in the OCX fraction, reflecting a lower phospholipid concentration. These values comparable to those reported for young bovine and human lens (Li *et al.*, 1987); suggest that the plasma membrane of the rat lens fiber cell is also very rigid. The high lipid content of the young cortical fibers relative to that of the embryonic fibers of the nucleus indicates changes in membrane composition during normal lens development.

The cholesterol concentrations reported here, ranging from 5.5 to 4 μg/mg lens dry weight, are considerably higher than those of 2.4 to 2.2 obtained by Cenedella (1984). Since the procedure of digitonide precipitation was employed in his study, it is possible that some of the cholesterol was not recovered. It is also likely that the weaned animals, having a body weight of approximately 65 gm at the beginning of our experiments, were much older than the pups from pregnant rats used in his study.

Serum cholesterol level in the galactose-fed rats

Plasma cholesterol levels in the rat remain rather constant during aging and drug manipulations. Only when the adaptive responses of the metabolic pathways are exceeded or blocked are changes in cholesterol levels found (Stange and Dietschy, 1984). The increase in the serum cholesterol level of rats on a high sugar diet suggests dramatic changes in hepatic metabolism. Since an elevated serum cholesterol level is known to inhibit *in situ* cholesterol synthesis required for cell growth and differentiation (Brown and Goldstein, 1980), the observed decrease in the total lens weight could be the result of an inability of the lens epithelium to form new fibers. The possibility that rat lens accumulate cholesterol from sources other than *de novo* synthesis has been suggested (Cenedella, 1982). Reports of morphological disorganization in the differentiating region of the lens epithelium of the galactose-fed rat (Cotlier, 1962; Gona,

1984) are consistent with this interpretation.

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REFERENCES

Bar-on, H. and Y. Stein (1968) Effect of glucose and fructose administration on lipid metabolism in the rat. *J. Nutrition* **94**: 95-105.

Broekhuyse, R.M. (1981) *Biochemistry of membranes. In*: Duncan G. (ed.) Mechanisms of Cataract Formation in the Human Lens. Acad. Press. London, UK. pp. 151-192.

Brown, M.S. and J.L. Goldstein (1980) Multivalent feedback regulation of HMG CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. *J. Lipid Res.* **21**: 505-517.

Cenedella, R.J. (1982) Sterol synthesis by the ocular lens of the rat during postnatal development. *J. Lipid Res.* **23**: 619-626.

Cenedella, R.J. (1984) Regional distribution of sterol and fatty acid synthesis in the ocular lens. *Exp. Eye Res.* **38**: 95-99.

Cotlier, E. (1962) The mitotic cycles of the lens epithelium. *Arch. Ophthal.* **68**: 801-809.

Gona, O. (1984) Cytoarchitectural changes in lens epithelium of galactose-fed rats. *Exp. Eye Res.* **38**: 647-652.

Keeting, P., D.-S. Dong, T.W. Lysz, *et al.* (1987) Rat lens prostaglandin biosynthesis during galactose-induced cataractogenesis. *Exp. Eye Res.* **43**: 1103-1110.

Kinoshita, J.H., L. Merola, K. Satoh, *et al.* (1962) Osmotic changes caused by the accumulation of dulcitol in the lenses of rats fed with galactose. *Nature* **194**: 1085-1087.

Kuwabara, T., J.H. Kinoshita and D.G. Cogan (1969) Electron microscopic study of galactose-induced cataract. *Invest. Ophthal.* **2**: 133-149.

Li, L.-K., L. So and A. Spector (1987) Age-dependent changes in the distribution and concentration of human lens cholesterol and phospholipids. *Biochim. Biophys. Acta* **917**: 112-120.

Li, L.-K. and L. So (1987) Age-dependent lipid and protein changes in individual bovine lenses. *Curr. Eye Res.* **6**: 599-605.

Lloyd, B., J. Burrin, P. Smythe, *et al.* (1978) Enzymatic fluorometric continuous flow assays for blood glucose, lactate, pyruvate, alanine, glycerol and 3-hydroxybutyrate. *Clin. Chem.* **24**: 1724-1729.

Reddy, V.N., D. Schwass, B. Chakarapani, *et al.* (1976) Biochemical changes associated with the development and reversal of galactose cataracts. *Exp. Eye Res.* **23**: 483-493.

Sokal, R.R. and F.J. Rohlf (1980) *Biometry.* W.H. Freeman and Co., New York. 859 pp.

Stange, E.F. and J.M. Dietschy (1984) Age-related decreases in tissue sterol acquisition are mediated by changes in cholesterol synthesis and not low density lipoprotein uptake in the rat. *J. Lipid Res.* **25**: 703-713.

Zlatkis, A., B. Zak and A.J. Boyle (1953) A new method for the direct determination of serum cholesterol. *J. Lab. Clin. Med.* **41**: 486-492.

半乳糖對大白鼠水晶體的蛋白及脂肪分佈的影響

李盧蝦 Raul Chiesa 唐榮山 傅守正

大白鼠用三種不同飼料餵養後，每個水晶體依其組織結構的强度，用機械力分解成皮層、中層及核心三部分。三種飼料是(1) Purina 白鼠飼料（對照組）、(2) 葡萄糖與 Purina 各半，(3)半乳糖與 Purina 各半。經十四天餵飼後，葡萄糖組白鼠水晶體乾重的增加較對照組白鼠爲少。半乳糖組水晶體則無顯著的乾重增加。在試驗的第四天，半乳糖組水晶體乾重與對照組已經有明顯的差異。在試驗的第一天，半乳糖組水晶球皮層部份乾重之比例增加已經顯著，證明水晶體組織已開始弱化。

在對照組中，水晶體膽固醇及磷脂含量皆由皮層向核心部位逐漸減少，其含量變化分別由 5.5 降至 4，及 15 降至 5 μg/mg 乾重。半乳糖組水晶體皮質之纖維細胞增加導致皮層脂肪總含量降低。水晶體中磷脂的成份是以磷脂乙醇胺、磷脂絲胺酸、磷脂膽素和飽和髓脂爲主。三種不同飼料餵食的白鼠，其水晶體磷脂成份均相似。半乳糖餵食組血清中膽固醇含量爲 72 mg/dl 高於對照組的 61 mg/dl。因爲血液高膽固醇含量抑制細胞成長及分化的作用，本試驗結果-半乳糖導致白鼠水晶體之減重-顯示半乳糖可能阻止或破壞水晶體發育及形成。